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THE MOTONEURONS OF THE SPINAL CORD OF THE FROG

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

1941

By

MAURICE L. SILVER

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THE MOTONEURONS OF THE SPINAL CORD OF THE FROG

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EIGHT TEXT FIGURES AND THREE PLATES (FIVE FIGURES)

INTRODUCTION

In the light of present knowledge, it seems that the most successful attack upon the problems of the central nervous system can be made by a combination of structural analysis with electrical methods.

The structure of the amphibian brain is probably better understood than that of any vertebrate group, thanks to the inspired efforts of Professor Herrick. The spinal cord, unfortunately, has never received such rigorous analysis. In order to apply such functional tests as localized electrical recording, or precise electrical stimulation, or strychninization, or determination of regional metabolic rate, the qualitative knowledge of nuclear masses, fiber-tracts, and neuropil regions must be supplemented by a quantitative knowledge of their exact position with respect to known landmarks, and of the numbers of neural elements involved. With this in mind, an investigation was begun in this laboratory, under the guidance of Prof. G. W. Bartelmez, on the central nervous system of the frog.

[1] This work was aided by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

I am indebted to Prof. G. W. Bartelmez for his encouragement and advice throughout the progress of this work, and to Prof. C. J. Herrick for his interest and help. I wish to thank Miss Agnes Nixon for her careful drawings of figures 1, 2, 3, and 8.

Rana pipiens was chosen as a representative amphibian because it was hoped that the anatomical findings might be correlated with the wealth of physiological experimentation on that animal. The scope of this problem, however, necessitated confinement of the analysis to the central nervous system caudal to the fourth ventricle.

MATERIALS AND METHODS

The material for this study consisted of sixteen complete series of sections of the entire central nervous system of the frog, fixed by perfusion, cut in the three standard planes and stained as follows: five Golgi, three Weigert, five Nissl, and three reduced silver [2].

The Golgi material was imbedded in celloidin and cut at 60 μ, the Weigert specimens were imbedded in celloidin and cut at 30 μ, the Nissl and reduced silver preparations were double-imbedded in celloidin-paraffin and cut at 15 μ.

These were supplemented by twenty-five partial series of sections of various levels of the spinal cord, cut frozen, in paraffin, or in celloidin, at thicknesses of 2 μ to 10 μ, and stained with iron hematoxylin, aniline acid fuchsin-toluidin blue, Mallory-azan, Benda's alizarin red-crystal violet, and also by reduced silver and reduced gold techniques developed in this laboratory for staining neuroglia.

All of the frogs chosen were healthy males, weighing not less than 30 gm. nor more than 35 gm., and having an overall-length (tip of snout to end of fourth toe) of not less than 195 mm., nor more than 205 mm.

One of these frogs, weighing 30 gm. and measuring exactly 200 mm. in length, was chosen as the specimen from which was constructed a chart of the position and number of all the motoneurons in the frog cord. Consequently, all the results hereafter described, must be considered to be particularly applicable to the "200-mm. frog."

[2] In October, 1941, I received many serial sections of the frog brain prepared by the Golgi, Weigert, Nissl and Cajal methods from Prof. C. Judson Herrick. Many of these contained levels far down into the spinal cord and were a valuable addition to the slides described. It is a pleasure hereby to acknowledge my thanks.

This animal was perfused through the heart with the following mixture: formalin 5 cc., acetic acid (glacial) 5 cc., 95% alcohol 70 cc., distilled water 20 cc. The entire nervous system was dissected out in the fixative and was carefully dehydrated over a period of 3 weeks, double imbedded in 3% Hercules celloidin-paraffin, and cut serially at 15 μ. This specimen was carefully measured in situ, and at all stages of the dehydration; the results were compared with the total number of sections cut, and the shrinkage for the entire neural axis (olfactory nerves to filum terminale) was found to be less than 3%.

Alternate slides were stained by the Nissl and reduced silver technique. Each one of 1060 sections was studied at a magnification of 400 diameters and the position of the spinal motoneurons plotted on millimeter-paper at a magnification of 50 diameters. Two plots were made, one a projection on the midsagittal, the other on the horizontal plane (figs. 9 and 10). Synaptic connections and other cytological details were drawn at a magnification of 1500 diameters (fig. 8).

On the basis of the charting of the motoneurons, microelectrodes were inserted into the cell columns at various levels and localized electrical stimulation applied. In order to determine the peripheral innervation, systematic electrical stimulation was carried out on lightly anesthetized, or decapitate frogs, using bipolar micro-electrodes of original design. These electrodes consisted of a 5-μ silver wire inside a glass capillary tube whose outer surface had been coated with silver, after it had been drawn to a 7-μ tip. In the earlier experiments, a Harvard inductorium, with 3 volts in the primary, was used for stimulation, but later a specially designed stimulator was used, which delivered "spike" shocks, the intensity and frequency of which could be varied independently. No attempt was made to determine absolute thresholds; the lowest intensity current was used that produced a visible muscular response.

The results of this investigation will be described in two parts: first, a qualitative account of the organization of the

frog spinal cord, including a description of the cell columns, cell types and connections; and second, a quantitative account of the numbers of neural elements involved.

PART I. DESCRIPTIVE ANALYSIS

The microscopic anatomy of the spinal cord is better appreciated against a background of the gross anatomy of the frog's central nervous system, described in detail by Reissner (1864), Stieda (1870), Ecker-Wiedersheim-Gaupp (1899), Ariëns Kappers and Hammer ('18) and Ariëns Kappers, Huber and Crosby ('36). A brief synopsis is introduced here to facilitate description.

The cord exhibits a brachial swelling (intumescentia cervicalis) and a lumbar swelling (intumescentia lumbalis) separated by a thoracic region (pars media); at its caudal end it tapers into a long filum terminale. From it emerge ten pairs of roots, numbered from two to eleven, the first having disappeared during development. The largest branch of N. II is the hypoglossal nerve, innervating the tongue. A smaller branch of II combines with III and IV to form the brachial plexus, innervating the arms. V, VI and VII innervate the thoraco-abdominal body-wall musculature; VIII, IX, X and XI form the lumbar plexus, innervating the legs.

In cross-sections stained by the Weigert method the level can be readily determined by consideration of (1) relative proportions of gray and white matter, (2) shape of the gray and white, especially the shape of the dorsal columns, (3) the position of the central canal, and (4) the presence of a dorsal fissure. Ecker-Wiedersheim-Gaupp (1899) and Kappers and Hammer ('18) distinguish only three levels in the spinal cord, but the apparent uniformity is only superficial, for on the basis of criteria that appeared during the charting of the motoneurons, at least ten segmental levels could be recognized.

The distribution of myelinated fibers in the frog cord is similar to that in mammals, the fibers being organized into dorsal, lateral and ventral funiculi. The distribution differs, however, in that the lateral columns in the frog do not extend

to the surface of the cord, as do the dorsal and ventral columns, but leave an area free of myelin sheaths under the pial membrane. This area extends from the emergence of the ventral root fibers to the entrance of the dorsal roots, where there is a typical "zone of Lissauer" and an extensive substantia gelatinosa Rolandi. The latter region was studied by Keenan ('29), but the lateral zone was not discussed, although it appeared in a drawing of lumbar cord. It should be emphasized that this lateral zone appears at all levels of the frog spinal cord from the calamus scriptorius to the level of the last neuron and it is approximately of the same size throughout.

Heavily myelinated fibers cross the midline in the ventral white commissure just above the ventral median septum. Above this, and just below the central canal, is the ventral gray commissure containing mostly unmyelinated fibers, and above the central canal are fibers of the dorsal commissure, finely myelinated, crossing through the central reticular area around the central canal in occasional strands, and more closely bunched just below the dorsal median septum.

The cellular constitution of the spinal cord, as seen in sections prepared by Nissl's method (toluidin blue-erythrosin), or stained with iron hematoxylin, can be separated under the usual divisions of (a) glial, and (b) neuronal elements. Under the former heading we can classify the ependymal elements lining the central canal, similar cells which have broken free from the central canal, but retain their foot of attachment to the pial membrane, similar cells which appear to be free of both the internal and external limiting membranes, and finally, small darkly-stained nuclei with almost no visible cytoplasm. Under the latter heading (b) we can classify the small neurons of the central reticular substance, distinguishable from the ependymal elements by their deep-blue staining cytoplasm, and the large motor-type neurons of the ventral horn, distinguishable from all the other cells in the spinal cord by their size, numerous dendrites, and large vesicular nucleus with its single nucleolus.

The reduced silver preparations confirm the differentiation between the small neurons and the large motoneurons, on the basis of their neurofibrillar constitution, and add the position of the unmyelinated fibers to the Weigert picture. The lateral zone is seen to be composed of fine unmyelinated fibers, which approach the pial membrane with the same radial orientation as the ependymal fibers, but then bend sharply and run more or less parallel to the surface of the spinal cord. The meshwork formed by these fine fibers outside the lateral columns is similar to the neuropil area just below the dorsal columns near the midline, and also to the fine feltwork of fibers surrounding the ventral horn cells. These fine fibers of the ventral horn end in small rings on the motoneurons (boutons terminaux) or in other cases, running directly along their dendrites with occasional varicosities (boutons de passage?) (fig. 8).

Of all the methods used, the Golgi has, of course, proved the most useful in determining the complete morphology of the elements seen with the other methods; it should be recalled that the Golgi preparations used in this investigation were fixed by perfusion, so that the details of synaptic regions must be assumed to have equal validity with results obtained by other metallic methods. In these preparations, the massive dendrites of the motor horn cells extend into the white matter, and their finer branches terminate in the sub-pial zone of the lateral funiculus, where they come into synaptic relation with axons and axon-collaterals, thus forming a true neuropil region, the "lateral neuropil" (called plexus circummedullaris by Cajal, 1891, and Sala, 1892). A similar zone appears within, and just under, the dorsal columns. Entering dorsal root fibers break up into terminal branches in relation to the dendrites and small cell-bodies of the dorsal horn, constituting the "dorsal neuropil." The lateral bundle of the dorsal root also sends fibers to the lateral neuropil where they come into contact with the dendrites of the motor neurons and the smaller interneurons (e.g., commissural and other neurons). It appears that Keenan's description of the substantia gela-

tinosa includes the dorsal neuropil and part of the lateral neuropil described above.

The Golgi material gives further evidence for the cell types indicated in the Nissl and reduced silver preparations: The motoneurons in the different cell columns, the interneurons connecting incoming axons with the motoneurons of the ipsilateral and contralateral sides, the dorsal horn neurons, whose processes cross in both the dorsal and ventral commissure, and the neurons of the middorsal gray matter which are the presumed cells of origin for the long ascending tracts in the spinal cord. And especially, the Golgi material demonstrates those neurons which send their dendrites and axons into the lateral neuropil.

Cell types

In the spinal cord of the adult frog many of the older authors found it difficult to distinguish between neuronal and glial elements; if the tissue were as undifferentiated as implied by this problem, then a detailed classification of neuronal types would be arbitrary. However, the details seen in the various preparations can frequently be correlated and permit the establishments of certain categories.

1. The ventral horn motoneuron. Like the homologous cell in mammals, this is the most characteristic cell in the frog spinal cord (figs. 4 and 5). It has a large vesicular nucleus containing a single nucleolus, and a perikaryon which in Nissl preparations may have a long diameter of 100 to 120 μ. The full size of these elements, however, may be appreciated only in Golgi preparations, in which the massive dendrites of the cell extend 300 to 400 μ through the white matter to the lateral neuropil, 500 μ into the dorsal neuropil, or even a millimeter into the contralateral half of the spinal cord, while the axis cylinder traverses 30 to 80 mm. to the musculature of the limbs.

These cells contain coarse Nissl bodies (figs. 1 and 3), smooth neurofibrillae (fig. 2), filamentous mitochondria, and a dispersed Golgi apparatus. Some of the cells contain a

melanin-like pigment (fig. 1) but these pigment bearing cells always appear at the periphery of the gray matter, most often in the ventrolateral portion of the ventral horn; it is possible that this may be correlated with the decreased vascularity of the funicular portion of the frog spinal cord. (A report on the vascular pattern of the frog's spinal cord is now in progress.)

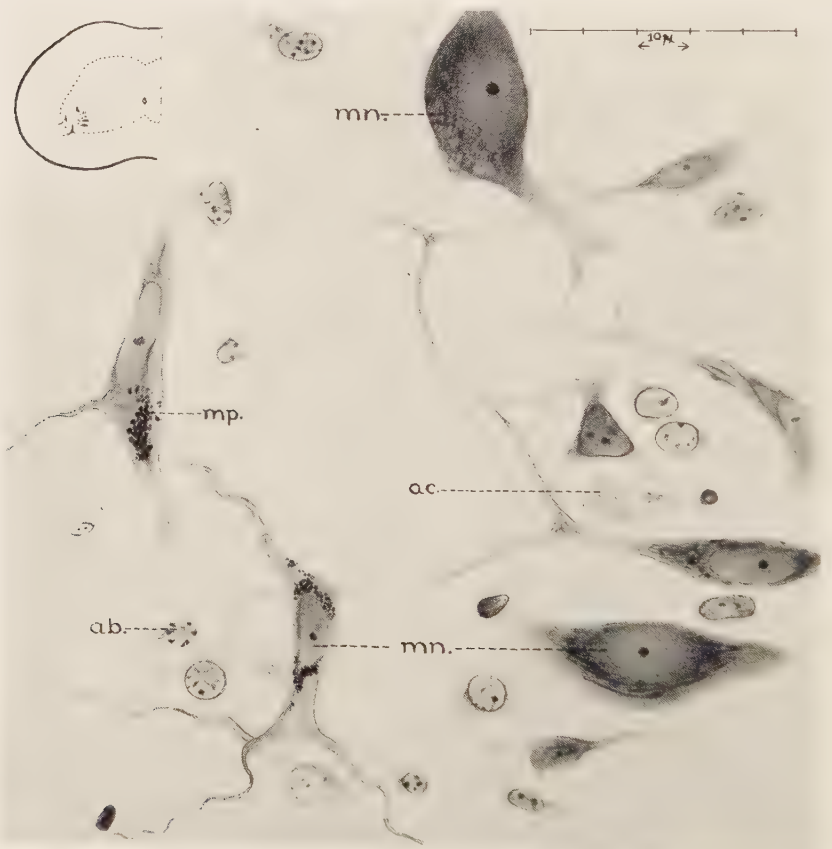


Fig. 1 A drawing from a transverse, 15-μ, double-imbedded section of frog spinal cord at the caudal end of the brachial plexus, stained with toluidin blue-erythrosin. The key figure at the upper left indicates the position of the pigment-containing neurons at the periphery of the ventral gray. ab., astroblast; ac., cluster of astrocytes; mn., motoneurons; mp., melanin-like pigment in peripheral motoneurons.

The actual shape of the ventral horn perikaryon varies considerably. In the hypoglossal nucleus (included in this description because the axons emerge in the brachial plexus) the cell body is ovoid (in Nissl preparations, fig. 3), although

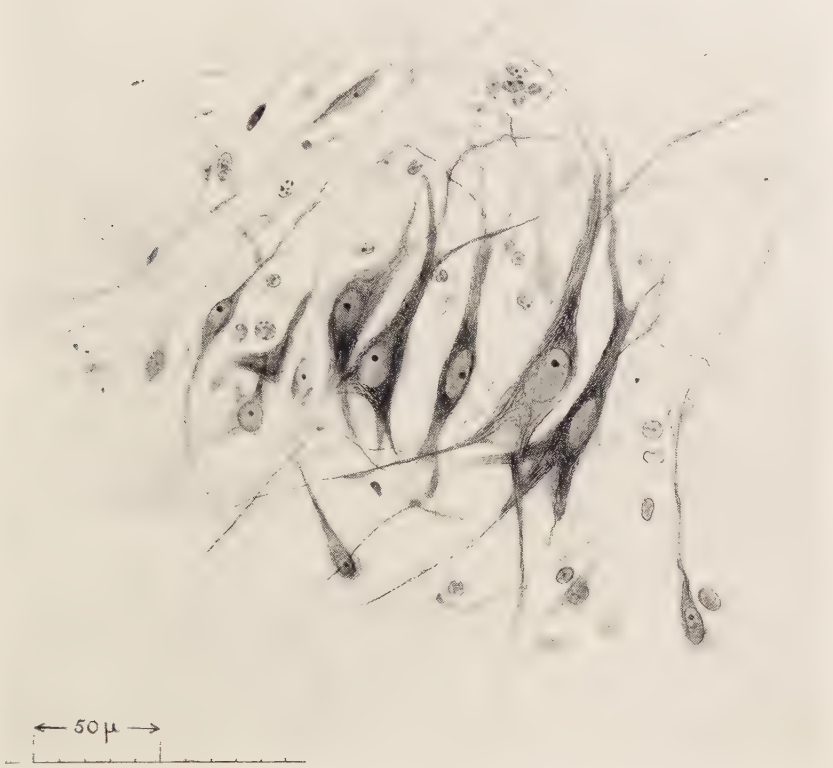


Fig. 2 A drawing from a transverse, 15-μ double-imbedded section of brachial spinal cord, stained with reduced silver to demonstrate neurofibrils. These motoneurons innervate the flexors of the arm.

the majority of ventral horn cells are multangular or spindle-shaped (figs. 1 and 2). Despite careful study, it has not been found possible to differentiate among the ventral horn cells on the basis of distribution of Nissl substance. All of the cells have the deeply-stained, large Nissl granules which are so

characteristic of the mammalian motoneurons. Probably the most important feature of the ventral horn motoneuron is the large surface offered for synaptic connections (figs. 5 and 8). This will be discussed at greater length in the section on "terminations" (p. 22).

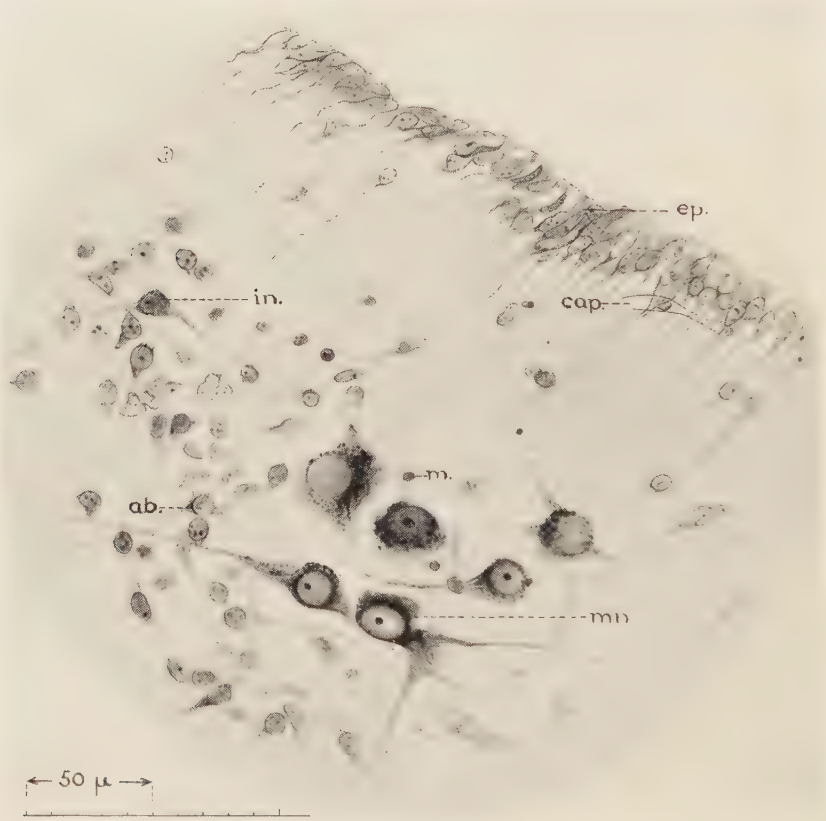


Fig. 3 Drawing from a transverse, 15-μ, section through the caudal end of the hypoglossal nucleus, at the caudal end of the fourth ventricle. ab., astroblast; cap., capillary; in., interneuron; m., microglia nucleus; mn., hypoglossal motoneuron.

2. The interneuron. This title would include most of the small neurons of the periventricular gray, but this term should be especially reserved for the cells which clearly serve as links in the chain of the local reflex. These small, conical

cells, with more dispersed Nissl substance and scanty neurofibrils, are most frequently found midway between the central canal and the lateral margin of the cord at the level of the central canal. In this position the interneuron which appears most frequently has three to five processes, and these usually are approximately equidistant from one another (fig. 6) extending into the lateral neuropil, the dorsal horn, and even

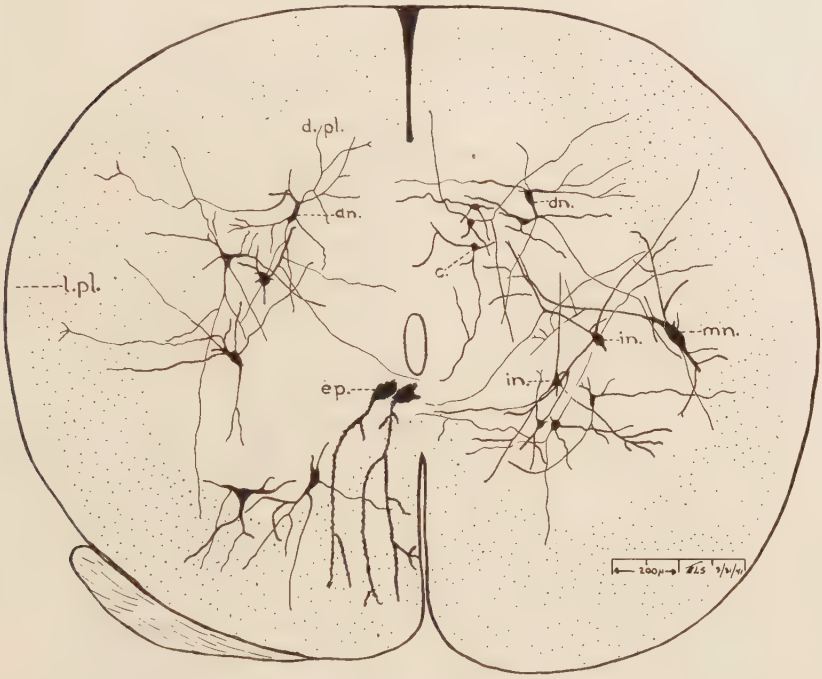


Fig. 4 A drawing of a transverse, 60 μ thick Golgi preparation, showing in a single fortunate specimen, the main cell types of the frog's spinal cord. c., commissural neuron; dn., dorsal horn neuron; d.pl., dorsal neuropil zone; ep., ependymal cell; in., interneuron; l.pl., lateral neuropil zone; mn., motoneuron.

into the contralateral half of the spinal cord. In the latter case, our "interneuron" would include the "commissural neuron" of the older authors; this latter term is, in my opinion, too inclusive, because almost every neuron in the frog spinal cord has some process that crosses the midline, and the axon is often very difficult to identify (only 1% of the moto-

neurons counted have clearly defined axon hillocks in Nissl preparations, or morphologically unquestionable axons in the Golgi). It is perhaps better to reserve the term commissural neuron for the cells close to the central canal which have relatively small dendrites and a single axonic process which crosses in either the dorsal or ventral gray commissure (axons

Figs. 5, 6 and 7 Higher power views of the cell types seen in the previous figure, showing detail of the extensive cell processes. Fig. 5, Motoneuron; Fig. 6, Interneuron; Fig. 7, Dorsal horn neuron.

are finely myelinated) to terminate among the cells of the contralateral ventral horn. The nuclei of these cells are very similar to those of the astroblasts and ependyma cells of the frog cord.

An irregular type of cell which might be classed with the interneurons, is the cell which sometimes appears just under

the lateral neuropil, far removed from the mass of the ventral gray. This type of neuron, which perhaps has migrated under neurobiotactic influences toward the lateral neuropil, still appears to mantain synaptic connection with the ventral horn.

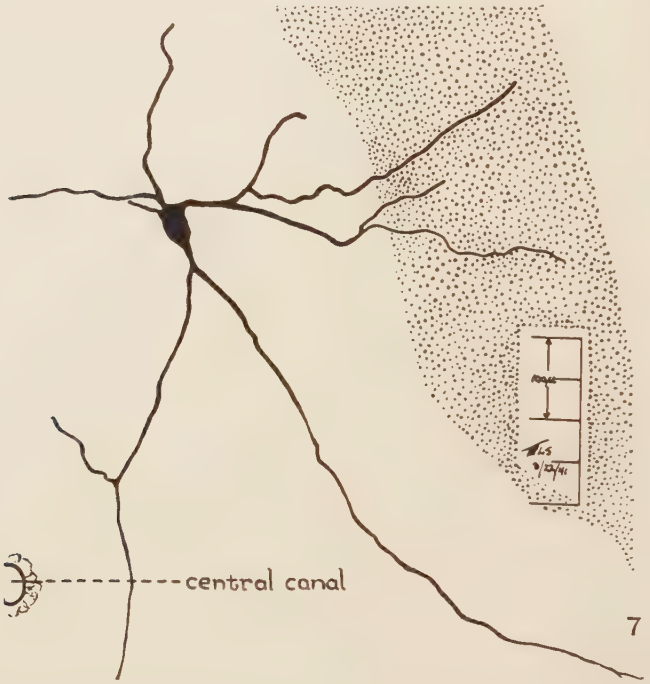
3. The dorsal horn neuron. There are certain differences, besides that of location, between these cells and those described

6

previously. Some of them, especially at the brachial plexus level of the cord, are as large as the ventral horn montoneurons, but the majority of the cells are a little larger than the interneurons (fig. 7) and differ from the latter in the distribution of their processes. Some of the dorsal horn neurons have processes which have been followed for 1.5 to 2 mm. in sagittal and horizontal sections, which with their collaterals, would

provide the anatomical basis for Dun's ('41) electrical findings of dorsal root potentials in spinal roots at a distance from the stimulated root.

In three Golgi preparations cells were seen in the dorsal horn (i.e., above the level of the central canal) which sent axons out in the ventral roots. These might be assumed to be the cells of origin of the sympathetic system in the frog, but they are too few in number, and on the basis of the quantitative



data which are given in the latter half of this report, it would appear that many of the preganglionic sympathetic neurons are morphologically indistinguishable from the small motoneurons.

I have not actually seen a dorsal horn neuron whose axon emerged in a dorsal root, although there have been many suggestive pictures in Golgi preparations, but I have actually seen cells resembling dorsal horn neurons (not typical spinal

ganglion cells) in the dorsal root half-way between the spinal cord and the spinal ganglion of nerve III. The connections of these cells are obscured by the darkened myelin sheaths of the entering fibers, but it is possible that we are dealing here with another example of neurobiotactic migration, and that these cells are entering into synaptic relationship with the dorsal root fibers, just as are the orthodox dorsal horn neurons with processes in the dorsal neuropil zone.

It is clear that these simple categories of motor and associated neurons leave unlocalized the cells of origin of specific fiber tracts. Except for the above categories, the remaining neurons of the spinal cord consist of a heterogeneous mass of small cells with irregular processes, which may or may not cross in the ventral white commissure, and whose axons may extend the entire length of the spinal cord to form the spinocerebellar or spino-bulbar pathways, or extend only 1 to 2 segments to form the shorter intraspinal paths. It is clear, then, that the concept of some authors that the size of the perikaryon as seen in the Nissl preparations is an indicator of the length of its axis cylinder, does not apply to the neurons of the frog's spinal cord.

Motor columns

The organization of the motor columns in the frog cord is of special interest, because the frog seems to be the first animal in phylogeny in which clearly defined motor neurons are organized in linear groups within a distinct ventral horn. It is to be hoped, therefore, that the functional pattern elucidated for the frog will prove to be fundamental to the more elaborate columnar organization of the higher vertebrates.

It is very difficult, even with careful study of serial sections of spinal cord, to determine the exact pattern of a cell column by piecing together a series of images. For this reason the brief generalizations of Ariëns Kappers and Hammer ('18) are not complete. An objective method, such as the plotting of all the motoneurons to scale, is adequate to describe this

pattern. For this purpose, a motoneuron was defined as a cell having a nucleus of at least 10 μ in its longest visible diameter, containing a single nucleolus, and whose perikaryon in any section studied had one diameter of at least 20 μ.

This arbitrary definition of a motoneuron included all the large ventral horn cells, which in the Golgi preparations were seen to originate ventral root fibers and excluded the small neurons of the central reticular substance.

The reliability of this criterion is confirmed by the closeness with which the total number of counted ventral horn cells approaches the total number of fibers in the ventral roots. This will appear in the description of quantitative results.

Briefly, the cell columns appear as follows: Caudal to the closure of the fourth ventricle near the midline, the hypoglossal nucleus tapers to an end. In the gray matter at this level, between the calamus and the emergence of the II nerve, the large neurons are diffusely situated in a formatio reticularis. As the cord enlarges into the brachial swelling, two columns appear, a smaller medial, and a larger lateral. Before the level of the III nerve, the lateral column can be seen to consist of two divisions, a dorsal and a ventral. Throughout the brachial plexus region, then, there is a ventromedial column, a dorsolateral column and a ventrolateral column. At the level of the IV spinal nerve, externally the brachial swelling tapers into the pars media, and internally, the two lateral columns taper into the medial column. This medial column is larger than it was at the brachial plexus level, and is more diffuse than the compact lateral columns of the higher level. This continues through the levels of the V and VI nerves, but at the level of the VII nerve (and accompanying the external lumbar swelling) the medial column moves laterally into a smaller, compact lateral column. At the level of the VIII nerve this column becomes larger, and at the IX level, has attained its maximum size. At the level of X, the lateral column becomes smaller and there is a suggestion of a medial column. By the level of the XI nerve both have begun to

taper; a short distance farther the cells have become more and more scattered, and finally the last neuron is seen, amid the glial reticular substance of the filum terminale.

The significant features of the columnar organization are: The presence of the medial column throughout the cord except for the lumbar enlargement; the presence of the lateral columns in the limb plexus regions only; the constant presence of neurons, i.e., the absence of a visible neuromeric arrangement.

This latter fact emphasizes that motoneurons situated between two ventral roots must send their axons for some distance cranial or caudal before emerging. It can be readily understood then, that even if a muscle or muscle group is innervated by a compact column of cells within the spinal cord, stimulation of peripheral nerve roots might convey the impression of an extensive intraspinal origin of motor fibers because of fortuitous emergence of the axis cylinders in more than one ventral root. For the frog, Sherrington's dictum (1892): "The positions of nerve cell bodies are at the same segmental level as the point of emergence of the nerve fibers they originate," does not apply. This consideration may be pertinent to the controversy over mono- vs. plurisegmental innervation of skeletal muscle (de Boer, '26, Kurkowsky, '35, et al.).

In order to determine the spatial relationship between the motoneurons and the skeletal musculature, electrical stimulation was applied as described above under "methods." The results of thirty-eight separate experiments, proceeding caudally down the spinal cord, were as follows: The hypoglossal nucleus in the frog is an uncrossed nucleus. With tongue everted from the mouth cavity, systematic stimulation of the length of the nucleus showed that the proximal musculature of the tongue (referred to its point of attachment in the mouth) was innervated by the cranial portion of the hypoglossal nucleus, while the distal portion of the intrinsic tongue musculature was innervated by the spinal portion of the nucleus. The line of demarcation between the muscle masses

was fairly sharp, and the experiments indicated that there was a proximo-medial portion and a disto-lateral portion which contracted in that sequence as stimulation proceeded toward the formatio reticularis. Neither single nor repetitive stimulation of a single area of the hypoglossal nucleus ever gave a complete tongue movement, such as flipping it back into the mouth cavity; this suggests that the movement of the tongue in securing food depends upon a complex temporal sequence of stimuli, probably arriving from the tectum. The presence of sensory fibers in the hypoglossal nerve was indicated by the fact that peripheral stimulation of the nerve with three successive shocks (never one alone) per second was able to initiate a complete swallowing movement. Stimulation of the hypoglossal nucleus itself never evoked such a reflex.

Attempted stimulation of the tractus solitarius did not yield constant results; in some cases there appeared retraction of the tongue, in others, blinking of the ipsilateral eye, and in others, no response — except when the strength of stimulus became so great that there was obvious spread to the arm muscles. Histological checks (after fixation with the electrode in situ) in five cases indicated that the variable response was due to the variable position of the electrode, but that in no case was the tip of the electrode more than 200 μ away from the tract. In general, stimulation of the formatio reticularis yielded inconstant results. In two experiments, stimulation of the ventral gray just beyond the closure of the fourth ventricle yielded extension of the hind limbs, without contraction of any intervening muscles.

Stimulation of the brachial plexus level of the spinal cord yields more constant results. The first limb movement obtained caudal to the hypoglossal nucleus is an adduction of the scapula; this is followed (as the stimulating electrode is placed at the level of the III nerve) by an inward rotation at shoulder joint, then flexion at the elbow and wrist with extension of the fingers, then between III and IV, extension of the arm at elbow and wrist with flexion of the fingers. At the

level of the IV nerve and beyond, contraction of the abdominal musculature occurs.

With the electrode within the plexus region of the spinal cord, it is possible to decrease the strength of stimulus, so that only a single muscle in the arm contracts. This is true of almost all the muscles, except the finger muscles. In no instance was contraction obtained in a single finger muscle without response in the others.

The obvious intraspinal localization obtained in the above experiments merely indicated that localized response could be effected by artificial stimulation, but shed no light on the question of whether discriminatory response did occur in these animals, whose behavior is usually described in terms of "mass response." According to Polyak's ('24) investigations on the spinal cord of Chiroptera, the lateral neuropil zone provides an anatomical basis for the integration of large muscle masses which must act in unison (e.g., in flying, or in swimming). But, in our experiments, stimulation of the lateral columns caused movements of single muscles. The threshold for stimulation was lower on the lateral surface of the cord than on either the dorsal or the ventral funiculi and suggested that stimulation of the dendritic arborizations of the motoneurons was taking place rather than direct stimulation of the perikarya. If axons in this region had extensive synaptic conections with the dendrites of many motor units that were adequate to discharge them, we might expect a more widespread muscular response. In any case, it should be emphasized that the lateral neuropil region of the frog spinal cord provides an instance where synaptic junctions are less than 20 μ from an intact surface that is readily available to an investigator; that it provides an ideal locus for testing various ideas about synaptic transmission, especially with respect to application of chemicals that might be involved in facilitation or inhibition of synaptic transmission; and that it provides a ready means of determining electrical changes concomitant with the transmission of the nerve impulse at synaptic junctions, without injury to the nervous elements involved.

In the pars media of the spinal cord, stimulation of the gray matter near the midline (electrodes applied from the exposed ventral surface of the cord) caused contraction of the dorsal musculature of the vertebral column, i.e., M. longissimus dorsi, etc., while stimulation at the same level, but in a more lateral position, caused contraction of the ventrolateral musculature of the trunk, i.e., M. transversus and M. externus obliquus.

Analysis of the results of stimulation in the lumbar region was very difficult because no means of compound electromyographic recording were available, and the heavy musculature of the thigh made it probable that individual muscles in the thigh were contracting without being visible from the surface. The following conclusions, however, may be drawn from the experiments: The motoneurons for the extensor muscles are located caudal to those for the flexors; the neurons of the adductors are located caudal to those for the abductors; localized stimulation can cause contraction of a single muscle (e.g., stimulation at the level of emergence of the IX dorsal root caused contraction of the tibialis anticus longus); and, except for occasional contraction of the M. obturator internus and posterior fibers of the obliquus externus, no portion of the body wall was seen to contract during stimulation of the lumbar spinal cord.

The results of electrical stimulation may be summed up as follows: The motoneurons of the medial column innervate the dorsal midline musculature. The motoneurons of the lateral columns innervate the limbs, the ventrolateral ones innervating the muscles of the proximal portions of the limb, the dorsolateral ones innervating the distal portion of the limb. The absence of the medial column in the lumbar region may be correlated with the presence of the filum terminale, as factors stemming from the absorption of the caudal musculature during metamorphosis.

The motoneurons may be stimulated within the gray matter at the level of their perikarya, within the white matter in the dorsal neuropil, or within the subpial lateral neuropil. In all cases, suprathreshold stimulation resulted in localized contraction, often within a single muscle. We may conclude that

electrical stimulation with micro-electrodes is a reliable method for determining spatial localization of somatic motoneurons.

Attention should be called to the fact that the brachial motoneurons are slightly larger than the lumbar motoneurons. The longest axis-cylinder to the arm is only three-eighths as long as the longest axis-cylinder to the leg, and therefore, in the frog, no necessary relationship exists between size of the perikaryon and length of its axon.

Terminations

The intimate association of nerve terminations of other cells with the motoneuron should justify a few remarks on the synapse in the frog; especially since no figures of synapses, to my knowledge, have appeared in the literature on the frog.

Figure 8 is drawn from an 8-μ section of lumbar spinal cord, from the ventral horn at the level of the X spinal nerve. This animal was perfused through the heart with 10% neutral formalin in frog Ringers' solution, the spinal cord cut serially, and stained with the protargol-Rochelle salts reduced silver method.

The fine fibers end on the perikaryon, dendrites or axon in small boutons, 1 to 3 μ in diameter, or in larger ovals, with a long diameter of 8 μ or less, and a short diameter of 3 μ or less. There are also occasional tulip-shaped or conical endings which are much larger, and bear the same size relationship to the other endings as do the club endings to other endings on the Mauthner cell in the teleost brain (Bartelmez, '15).

The various histological methods used on the central nervous system of the frog, Rana pipiens, furnish evidence of protoplasmic discontinuity at the synapse similar to that observed in other forms (cf. Bartelmez and Hoerr, '33).

It should be noted that the number of endings seen on a single cell in a thin section stained with reduced silver, is rarely more than twenty, whereas in similar ventral horn cells in mammals the number counted may be hundreds. There are two possibilities to account for this difference. First, that in

my preparations we are dealing with incomplete staining of these small histological elements, and that actually the ventral horn motoneurons in the frog are almost entirely covered with terminations as are the homologous cells in mammals. The other possibility is, that actually, the synaptic field around the perikaryon is relatively small as compared with the neuropil zones at the periphery of the spinal cord, where there are manifold potentialities for neural transmission.

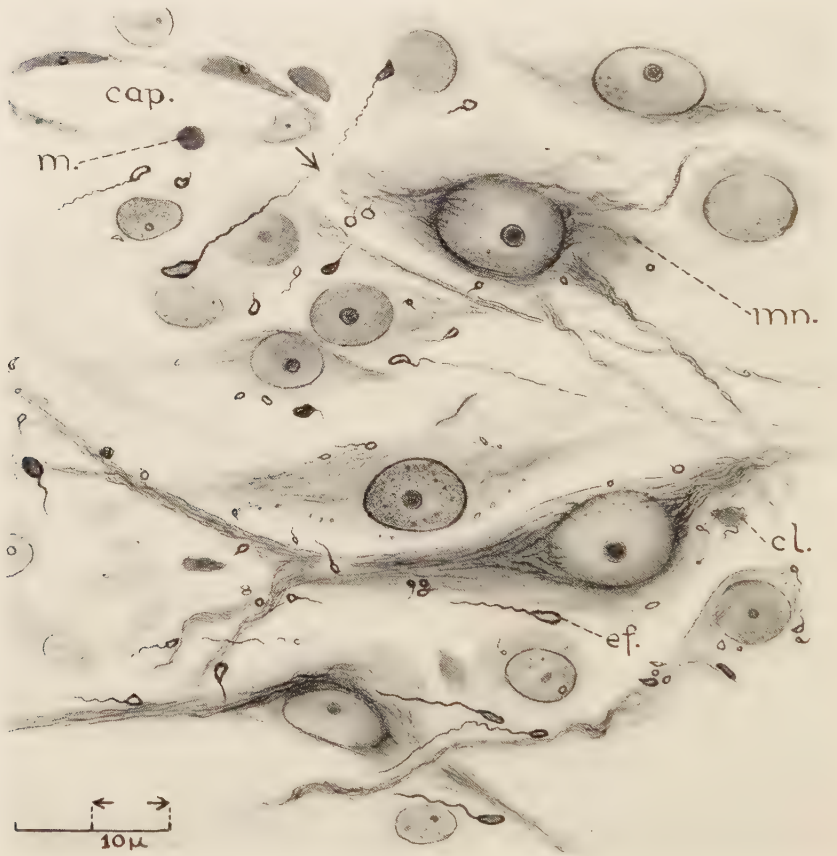


Fig. 8 A drawing from an 8 μ, double-imbedded, transverse section of lumbar cord, stained with the protargol-Rochelle salts reduced silver method, showing details of the synaptic terminations on the motoneurons. cap., perfused capillary; cl., club-type ending; ef., most common type of end-foot; m., microglia nucleus; mn., motoneuron.

I would favor the latter interpretation, because none of the methods used reveals more detail than the brilliantly differentiated silver preparations; it would coincide with an evolutionary concept of the morphology of synapses, in which the primitive type, as we see it in the amphibian nervous system, is one of the free arborizations of both cells meeting in neuropil zones (Herrick, '34) and the advanced type is one of axonal endings contacting specific cells by means of specialized terminals.

The contact of a single axonal ending with a single motoneuron is rare, although this is the picture usually seen in thin sections. (Even in fig. 8, however, at point marked by the arrow, a single fiber is seen to divide and end on two different cells.) In Golgi preparations, examined with the oil immersion lens, individual axis cylinders can be seen to break up into grape-like clusters which end upon 4 to 5 motoneurons. Some of the attached fibers of these smaller end-bulbs have been traced, in a single section, to a triangular interneuron in the middorsal gray of the same side. The cell of origin of the larger, pear-shaped "club" ending has not yet been found.

Incoming dorsal root fibers have been traced into the dorsal and lateral neuropil zones, and oil-immersion study at 1500 diameters reveals their ultimate terminations as free arborizations which resemble the afferent portion of an olfactory or cerebellar glomerulus. These terminal branchings frequently have blebs, and it is difficult to determine whether these comprise their actual form or whether they are caused by the fixation of the tissue, despite the fact that this was performed in all cases (including the Golgi) by perfusion.

If the finer structure of the synapse is to be of value in helping to understand the physiology of synaptic transmission, certain simple concepts which have developed out of previous studies must be replaced by mechanisms which consider the elaborate structure which is more completely seen with the Golgi method. That is, the classical concept of the motoneuron (and other neurons) is that of a funnel, quantitatively sum-

ming the excitatory processes brought in at the dendrites and perikaryon and, after the attainment of a certain threshold, discharging down the axis-cylinder. From the pedagogical point of view, this interpretation is adequate, and validly permits of the drawing of diagrams indicating paths of conduction in long pathways. But in short neuronal circuits, for example in intrasegmental spinal reflexes, this mechanism overlooks the fact that when one dendrite of a neuron is stimulated, the excitatory process spreads along this dendrite to the perikaryon, and then not only moves over the perikaryon toward the axis cylinder, but also sweeps back over the other dendrites, and could therefore leave these other dendrites refractory to their normal source of stimulation. This phenomenon, occurring at the interneuron, by blocking this cell, could readily account for central inhibition. That is, both motoneuron and interneuron have dendrites in the lateral neuropil zone, while the interneuron has axonal terminations on the motoneuron. The arrival of the excitatory state at the lateral neuropil would "fire" both the motoneuron and the interneuron. If we assume that the activity of the interneuron is necessary to maintain the discharge of the motoneuron, then the blocking of the interneuron, perhaps by an additional source of stimulation at another of its dendrites, may be adequate to effect inhibition.

Such considerations would place emphasis upon the spatial organization of the neuron as a factor which is of importance similar to the temporal arrival of impulses in the integration of the activity of the central nervous system.

PART II. QUANTITATIVE ANALYSIS

The need for a quantitative approach in an effort to solve some of the problems of the nervous system was probably first recognized by Stilling (1859), but the obvious difficulties in preparing long series of sections and counting thousands upon thousands of neural elements have kept this branch of neurology relatively unexplored.

If the frog has received more than a proportionate share of attention in these quantitative studies, it is largely attributable to the pioneer work of Birge (1882). This investigator, working in Carl Ludwig's laboratory, counted neurons and nerve fibers in seven frogs. Although the methods he used were block staining methods involving osmium tetroxide and carmine, so that he could not count the unmyelinated fibers and had difficulty in differentiating the smaller neurons, his counts were so accurate that they compare directly not only among themselves, but also with all the enumerations on frog made since that time, including the work in this paper.

The most reliable criterion for an accurate count of motoneurons in the frog's spinal cord is the close correspondence of the results of counting on the right and the left sides. If the enumeration is done objectively, i.e., if the numbers obtained from counting the individual sections are not totaled until at least 500 sections are counted, the difference between the results on each side should not exceed ½ of 1%.

In Birge's counts, a frog weighing 22 gm. had 3385 motoneurons in the right half of the spinal cord and 3375 motoneurons in the left half; and a frog weighing 63 gm. had 4272 motoneurons in the right half of the spinal cord and 4267 motoneurons in the left half. In the spinal cord of our specimen, weighing 30 gm., there were 3546 motoneurons in the right half of the spinal cord and 3542 motoneurons in the left half.

This suggests a relationship between body weight and number of spinal motoneurons at least within certain limits. With body weight in grams as the abscissa, and number of motoneurons as the ordinate, it is possible to construct a graph which is a surprising approximation to a straight line. The formula representing this graph, where X is the body weight in grams, and M is the total number of motoneurons in the spinal cord, is: $M = 5750 + 45\,X$.

Thus, in predicting the number of motoneurons in the 30-gm. frog, $M = 5750 + 45\ (30) = 7100$. The actual number counted is 7088, a difference between the predicted and counted

values of less than 2 motoneurons per thousand. When this formula is applied to Birge's 22-gm. and 63-gm. frogs, the predicted value differs from the counted value by 3 and 5 motoneurons per thousand respectively, or less than $\frac{1}{2}$ of 1%. This suggests that species difference (Birge used Rana temporaria, we used Rana pipiens) is not a disturbing factor.

It is not established that weight is the only variable that is correlated with the number of motoneurons in the frog spinal cord, and for that reason all the frogs used in this report were carefully controlled as to sex, weight, total length, length of body (tip of snout to cloaca) and length of femur, following Donaldson (1898) and Donaldson and Schoemaker ('00), so that these data will be available for re-translation in terms of more exact correlating values if these are ever established.

Donaldson ('02) published a formula for determining the weight of the central nervous system of a frog from values for the animal's weight and total length. Hatai ('11) published a formula relating weight to length in the frog and, if valid, it would reduce Donaldson's formula to a single variable. However, Hatai did not describe the number of animals used in preparing his curve; in fact, it appears that he merely re-worked some of Donaldson's data (and Donaldson used sixty-two selected measurements out of a total of eighty-eight observations on male and female frogs). From fifty of my own measurements, all made on male frogs (eliminating the error of weighing females whose eggs and ovaries have been removed) kept at the same temperature (thermostatically controlled) and under identical conditions of hydration, it appears that weight and length of a frog are independent variables. But at the present time, as a first approximation and pending further data, the formula given above is adequate to describe the relationship between weight of a frog and number of spinal motoneurons, at least if the weight is between 15 and 75 gm.

The following facts about the counting should be emphasized: It is essential that reliable landmarks be employed in describing the limits of the spinal cord, because of its con-

tinuity with the medulla oblongata. The caudal limit is the position of the last motoneuron within the spinal cord, the cranial limit in this study is the beginning of the fourth ventricle. But the transition of the ventricle into the canal has a definite cranio-caudal extent, and the starting point for the counting should be the point at which the central canal has narrowed to its definitive size for the remainder of the cord; this point corresponds with the caudal end of the hypoglossal nucleus, and is a reliable internal point of reference that is free from the variability of the external closure of the ventricle. Between the cranial and caudal limits as here defined, the count of motoneurons is: Right, 3546, Left, 3542.

If the spinal cord is taken to include everything to the point where the lateral walls of the fourth ventricle meet in the midline, thus including a large portion of the hypoglossal nucleus, the count is: Right, 3924, Left, 3932 motoneurons.

Figures 9, 10, 11, and 12 are projections upon the horizontal and sagittal planes of the motoneurons of the frog spinal cord. The original plots were made at a magnification of 50 diameters, and this has been reduced to 25 diameters in reproduction. Each circle represents the projection on the appropriate plane of a single neuron except in those cases where two neurons were on the same perpendicular to the plane of projection.

If the number of neurons in the entire cord is divided into portions corresponding to brachial cord, thoracic cord, and lumbar cord, and Birge's counts of motoneurons and ventral root fibers are also included, it is apparent that the brachial region of the spinal cord contains 52 to 56%, the thoracic region 8 to 12%, and the lumbar region 34 to 39% of the total number of neurons (fig. 13). Whatever the biological significance of this distribution, it is evidently opposed to the commonly held view that mass of peripheral musculature is directly related to number of motoneurons. This distribution suggests that the fore-limbs might be able to make muscular movements requiring greater discrimination or better co-or-

dination, but this has not been demonstrated by a functional test.

The segmental distribution of motoneurons is so clearly marked that the number of motoneurons in a 15-μ section (average of five sections) is a strong supporting criterion in determining the level of a given section within the spinal cord. In a frog having approximately 3500 motoneurons in each half of the spinal cord, table 1 would be an adequate description of their regional distribution:

TABLE 1

Distribution of motoneurons.

LEVEL OF NERVE	AVERAGE NUMBER OF MOTONEURONS PER 15 μ SECTION OF SPINAL CORD	AVERAGE NUMBER OF MOTONEURONS PER RUNNING MILLIMETER OF SPINAL CORD
II	4–5	300
III–IV	7–8	500
IV–V	5–6	350
V–VI	2	125
VII–VIII	3	200
VIII–XI	4–5	300
XI	1	60

The enumeration of these data has more than descriptive value; when compared with other quantitative neurological data, it may direct the investigator to seek out unsuspected mechanisms for neural integration. For example, Gaule (1889) counted the number of myelinated fibers at five levels of the spinal cord of a 60-gm. frog. According to previous counts of myelinated root fibers made by Birge, we could expect 10,600 myelinated dorsal root fibers and 8,500 myelinated ventral root fibers.

(The actual count of ventral root fibers made by Birge on a 63-gm. frog was: Right, 4272 and Left, 4267, or a total of 8539 ventral root fibers. The total number of ventral horn motoneurons as calculated from our formula would be $M = 5750 + 45\ (63) = 8585$, a discrepancy of about ½ of 1%.)

In this frog, in which there were a total of less than 20,000 myelinated root fibers, Gaule counted in a single cross-section more than 74,000 myelinated fibers (table 2).

TABLE 2

Excess of total myelinated fibers over total root fibers.

LEVEL OF NERVE	BIRGE'S COUNTS OF MYELINATED FIBERS IN		GAULE'S COUNTS OF MYELINATED FIBERS IN ENTIRE CROSS-SECTION OF SPINAL CORD
	DORSAL ROOT	VENTRAL ROOT	
II	124	783	56,674
III	1,649	975	74,699
IV	264	481	
V	193	106	41,825
VI	224	114	
VII	184	159	61,058
VIII	562	142	
IX	1,101	870	
X	993	441	16,313
XI	41	212	
Total	5,335 × 2 = 10,670	4,283 (one side) × 2 = 8,566	

All of the counts described above were complete counts, i.e. at no time was any sampling or approximation introduced by the figures. The question of the cells of origin for this tremendous excess of myelinated fibers remains to be answered.

The first possibility, of course, is descending fibers from the medulla. This undoubtedly contributes a fraction, but the number of fibers decreases in the caudal direction (Wallenberg, '07), while the number of myelinated fibers at the level of the VII spinal nerve is 10,000 greater than the number at the level of the fifth spinal nerve. This is more than can be accounted for by the relatively slight difference in entering dorsal root fibers or emergent ventral roots. The second pos-

sibility is that these fibers are the axis cylinders of the small neurons of dorsal horn or the central reticular area, but in the Golgi material, these axis-cylinders are seen to be mainly unmyelinated, crossing in the dorsal or ventral gray commissures. A third possibility is that entering myelinated dorsal root fibers divide more than once. If this occurred at the level of entry of the dorsal root, it should occasionally be visible in the sagittal or horizontal sections, and if it occurred at a higher level, there would be a greater count at level V than at level VII, neither of which is the case. This latter point also applies against the possibility of these fibers being largely long ascending ones. Another possibility is that the motoneurons may have more than one axon, or myelinated axon-collaterals. The presence of axon-collaterals in Amphibia is denied by von Lenhossék (1895), but we have seen them, not only in the Golgi, but also in the reduced silver preparations. Further, in the Nissl preparations and in reduced silver preparations, motoneurons were seen which appeared to have two axon hillocks and two processes which were anatomically indistinguishable from axons. If these observations are correct, and these intraspinal axons were myelinated, they might readily account for the increased number of myelinated fibers found by Gaule in the plexus levels and, further, they would suggest an integrative mechanism whereby a single neuron could discharge down its axis-cylinder to a muscle, and simultaneously discharge down an axon-collateral or a second axon to affect nearby or distant neurons. This would supplement the role of the interneuron in the integrative mechanism described previously, in which afferent impulses enter the spinal cord by way of the dorsal roots, and synapse with the dendrites of both the motoneuron and the interneuron in the marginal neuropil, and would again emphasize the conclusion that more than just one of the many neuronal processes are important in its integrative activities.

We hope that this work may lead to a better correlation of structure with function in the understanding of reflex activity of the spinal cord.

SUMMARY

1. The organization of the frog spinal cord is described, with especial reference to the cell types, synaptic terminations, and the columnar organization of the motoneurons.

2. The ventral horn motoneuron, the interneuron, the dorsal horn neuron, and other neuron types are differentiated. No relationship is found between size of perikaryon and length of its axis cylinder.

3. Synaptic fields are found at the periphery of the spinal cord (lateral neuropil), at the margin of gray and white matter (dorsal neuropil) and at the level of the perikarya. The type of termination found in the latter region resembles the boutons terminaux of mammals.

4. A medial column of motoneurons is present throughout the spinal cord and a lateral column appears only at the level of the limb plexuses. There is no evidence for a neuromeric arrangement of motoneurons.

5. The position of every motoneuron was plotted upon the horizontal and sagittal planes and the total number counted.

6. A formula is proposed for determining the total number of spinal motoneurons from the body-weight of the frog.

7. The function of the motoneuron columns at the various levels of the spinal cord was tested by electrical stimulation with micro-electrodes. The medial column was found to innervate the axial musculature, and the lateral column was found to innervate the limb musculature. Within the spinal cord, flexor motoneurons are cranial to extensor neurons, and adductor neurones are cranial to abductor neurons.

8. Some integrative mechanisms in the frog spinal cord are discussed in light of the histological findings.

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PLATE 1

EXPLANATION OF FIGURES

9 Plot of positions of motoneurons on the horizontal plane, each circle representing a single motoneuron, except where more than one cell is on the same line of projection. This figure represents the spinal cord of the frog from the IV ventricle to the V spinal nerve. Note the hypoglossal nucleus, the condensation of cells in the brachial plexus region, and the fusion of the smaller medial and larger lateral cell columns to form a single mass below the level of the brachial plexus. × 25.

10 Similar plot on the midsagittal plane. Note that the greater number of brachial plexus motoneurons are in the dorsal part of the lateral column. × 25.

PLATE 2

EXPLANATION OF FIGURES

11 Plot of position of motoneurons on the horizontal plane, each circle representing a single motoneuron, except where more than one cell is on the same line of projection. This figure represents the spinal cord of the frog from the V spinal nerve to the filum terminale. Note the absence of the medial cell column throughout the main part of the lumbar plexus and its reappearance at the caudal end of the lumbar enlargement. × 25.

12 Similar plot on the midsagittal plane. Note that there is no clear separation of the lateral column into dorsal and ventral portions, as is present in the brachial enlargement, and that the medial column, present at the cranial and caudal ends of this projection, is always more ventral than the lateral column. The cells above the central canal are large dorsal horn cells. × 25.

PLATE 3

EXPLANATION OF FIGURES

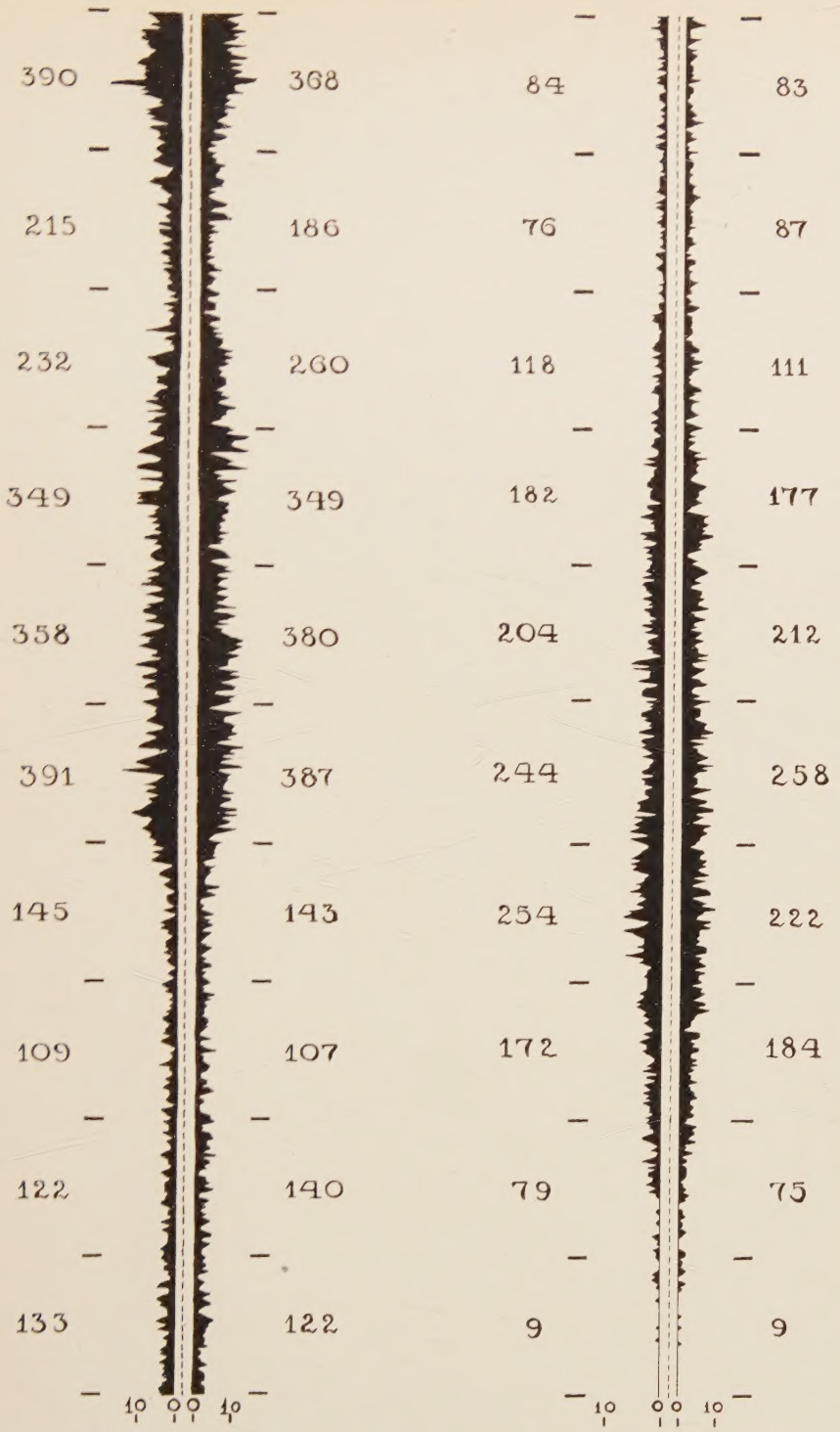
13 This chart represents the number of motoneurons at various levels of the spinal cord of the 30-gm., 200-mm. frog described in the text, from the IV ventricle to the filum terminale. The graph on the left is a representation of figure 9 and 10, and the graph on the right is a representation of figures 11 and 12.

The graph represents cell density on the basis of numbers of cells in each 15-μ section. The scale at the bottom of each graph represents 10 motoneurons.

The numbers along the sides of the graphs represent the count of motoneurons between each pair of horizontal marks, each length representing 0.75 mm. The graphs therefore, represent a total of 15 mm. of spinal cord, cut serially at 15 μ, each section giving one point to the right and left sides of a graph.

The totals for this chart are as follows: Left, 3866 motoneurons, and Right, 3860 motoneurons. The upper half of the spinal cord (from the hypoglossal nucleus to the V spinal nerve) contains: Left, 2444 motoneurons, and Right, 2442 motoneurons; the lower half (from the V spinal nerve to the filum terminale) contains: Left, 1422 motoneurons, and Right, 1418 motoneurons. This demonstrates a greater number of motoneurons in the brachial plexus region of the spinal cord as compared with the lumbar plexus.

The cranial limit of the spinal cord as defined in the text, i.e., at the caudal end of the hypoglossal nucleus, is 0.6 mm. from the upper limit of the left graph. From this point to the filum terminale, a distance of 14.4 mm., the count of motoneurons is: Left, 3542 and Right, 3546.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